SOME EXPERIMENTS CONDUCTED WITH PURE CULTURES OF BREAD YEAST

By William F. Henderson

Study of the conditions under which yeast colonies most rapidly develop, also of the conditions under which yeast produces carbon dioxide, have always been very interesting as well as practical. The failure to secure satisfactory yeast growths and gas productions when ordinary bacteriological media were used, led the author to pursue the problem more in detail, with the discovery of some very interesting facts concerning yeast development.

Isolation of the Yeast Colonies

Some pieces of "Yeast Foam" were mixed with distilled water. Agar and gelatin plates were made from diluted portions of this stock solution and the agar plates were incubated at 38° C. The gelatin plates were allowed to develop at room temperature, 20° C. The yeast colonies developed on the agar plate along with bacterial colonies, but none of the yeast colonies grew very large. The largest was not quite one mm. in diameter, circular, soft, and dull gray. The colonies on gelatin developed much more slowly and were no larger than those on agar.

Sub-cultures were made in sloped agar tubes. The maximum development gave a very thin streak of circular colonies, closely packed together. The development was scanty and far from satisfactory. Microscopical mounts were made from one of these streak cultures and no organisms were found to be present save the yeast. This pure culture was saved and was used as the source of material directly or indirectly, for all later experiments.

Experiments on Solid Media

The familiar relationship which exists between yeasts and sugar fermentation at once suggested the addition of sugar to the agar medium. This was tried as follows:

Author's Note: During the course of these experiments many helpful suggestions were made by Dr. A. A. Tyler to whom the author wishes to express his appreciation.

Small amounts of cane sugar, lactose, and glucose were added, respectively, to three agar tubes. These tubes were sterilized and cooled in a sloping position, and when cold, were innoculated from pure yeast cultures. The material was incubated for 24 hours at 38° C. and the results noted. The cultures on cane sugar agar and on lactose agar had scarcely developed at all; only thin, transparent streaks were visible. The yeast on glucose agar, however, had grown so rapidly that the entire surface of the medium was covered with the thick creamy-white mass of colonies. Upon microscopical examination, this material on glucose agar proved to be pure yeast culture. It would seem then, that the monosaccharides, of which glucose is an example, furnish much more favorable conditions for yeast development than do the disaccharides.

Further experiments were performed, using a larger variety of sugars. Seven agar tubes were prepared, six of which contained the following sugars, respectively:

Monosaccharides:	Disaccharides:
Glucose	Saccharose (cane sugar)
Galactose	Maltose
Levulose	Lactose

The seventh tube was used without sugar and served as a check.

One cc. of sterile water was poured into each of seven Petri dishes which had previously been sterilized. This water was innoculated with a small amount of yeast material and the plates poured in the usual manner, using the seven tubes of agar described above. These plates were incubated at 38° C for 28 hours. Development on two of the monosaccharides (glucose and levulose) was very rapid, while on the galactose and on the disaccharides, development was slight. Of the disaccharides, cane sugar seemed to furnish the most favorable conditions. The largest colonies on the glucose and levulose agar plates were surface colonies 6 to 7 mm. in diameter. The largest colonies on any of the other plates were 2 to 3 mm. in diameter. These occurred on the cane sugar plate and were surface colonies. The accompanying illustration shows the results obtained on these plate cultures. The plate containing galactose agar was not included in the photograph as it was identical in appearance with No. 6 (agar without sugar). It seems, therefore, that galactose, maltose, and lactose contribute very little, if any, to the conditions favorable for

yeast development on solid media; that cane sugar improves the conditions slightly, and that glucose and levulose produce conditions exceedingly favorable for yeast growth.

Variations in Morphology

When yeast grows in liquid media, the cells produced are of the familiar form viz., round, or more often, oval. As the budding occurs, small round or oval cells are produced, and these break off very readily, especially if the medium is agitated by shaking or stirring. In the case of the yeast used in these experiments, the maximum diameter attained by the cells in liquid media was from 7 to 9 microns. It is evident at the outset, that the conditions encountered by the yeast in or on a solid medium were entirely different from those encountered in a liquid medium. A careful examination of the plate cultures described previously showed that while the colonies were still minute, those on the surface, as well as those below the surface of the agar, were composed of cells identical in appearance with yeast cells grown in liquid media. As the colonies became older and larger, those on the surface retained their generally circular form, composed of ordinary oval yeast cells closely packed together. At the edge, the layer was one cell thick, but in the center budding had occurred vertically, thus rendering the colonies more or less opaque. Cells in all stages of budding could be seen, as the solidity of the medium held all the cells and buds in their respective positions.

As the deep, embedded colonies grew older, they attained a distinctly stellate appearance. These colonies possessed a small circular "nucleus" of ordinary oval yeast cells, but at the edge, some of the cells had become greatly elongated, growing in a direction away from the mass of the colony. Repeated budding and elongation of cells, together with the solidity of the medium resulted in the formation of long branched filaments, extending radially from the central colony. When cells from these stellate colonies were transferred to a slide and examined, it was found that the cells had all broken apart in the transfer. The cells observed were almost all single, some being oval in shape and others very long and slender. It is evident then, that the solidity of the medium (causing the cells to be immovable in reference to each other) was the cause of the filamentous formation. Furthermore, as the initial cells of the colony grew and multiplied,

a paucity of food would soon arise in the immediate vicinity. In liquid media, diffusion would maintain the supply of food for a considerable length of time, but in a solid medium diffusion would be slow.

As the young colony grew and spread in all directions, the food supply in the medium within the colony soon became almost exhausted. Very soon, those cells at the edge received the food stimulus only from the medium outside the colony. They responded to this more localized stimulus by growing and budding in a direction away from the mass of the colony. The location of the cell contents proved also to be interesting. The older, elongated cells were highly vacuolated. The younger, elongated cells (farther away from the mass of the colony) were less vacuolated. The vacuoles appeared first in the older part of the cell (nearer the mass of the colony) and the cell protoplasm tended to collect in the end of the cell away from the mass of the colony. Finally, the terminal cell contained only a few very small vacuoles and in many cases was found to be budding at the free end. The occurrence of more than one bud resulted in the formation of a "branch" in the filament. The accompanying figures show some of the types of filamentous growths and elongated cells.

Experiments on Gas Formation

A Smith gas tube was filled with 1% lactose bouillon and innoculated with pure yeast. Development occurred in the open arm only and no gas was registered in the tube. This suggested the thought perhaps the yeast was aerobic in character and would not develop to any extent under anaerobic conditions.

Gas tubes were prepared with two-hole cork stoppers; through one hole was placed a glass tube drawn out to a fine capillary and reaching to the bottom of the gas tube. Through the other hole was placed a short glass tube to allow exchange of gases as changes of pressure required. Both tubes were bent down outside and plugged with cotton. The gas tubes were filled with sugar bouillon, corked, and sterilized. The tubes were carefully innoculated with pure yeast and then from 30% to 40% of sterile oxygen forced into the closed arm through the capillary. A check tube was prepared in which no gas was admitted. These tubes were incubated 120 hours after which time no further changes seemed to occur. The residual gas in

the tubes was tested for carbon dioxide by absorption in 5% potassium hydroxide solution. The results were as follows:

Media	Initial Gas	Final Gas	% Absorbed by KOH	Residual Gas
Bouillon+ 1% Cane Sugar	Oxygen 38%	8%	5%	3%
Bouillon+ 1% Lactose	Oxygen 34%	6%	3%	3%
Bouillon+ 1% Glucose	Oxygen 35%	8%	0%	8%
Bouillon+ 1% Lactose	Air 32%	27%	3%	24%
Bouillon+ 1% Lactose	No Gas	No Gas	—	—

Where oxygen or air was admitted, development occurred in both arms of the tubes. In the last tube where no gas was admitted, development occurred in the open arm only. These tests strongly suggest that the yeast used (ordinary bread yeast), is aerobic. This conclusion is supported by the fact that in the agar plates described earlier, the deep colonies never attained any great size while the surface colonies grew (when on suitable media) to a large size.

According to chemical laws of gases, one volume of oxygen will produce the same volume of carbon dioxide. However, when we consider that carbon dioxide is 25 or 30 times as soluble in water as is oxygen, the shrinkage of gas volumes in the tubes is easily explained. As the slightly soluble oxygen was replaced by carbon dioxide, this latter gas would remain dissolved in the water and the liquid would rise to replace the oxygen used. This could proceed until the liquid became practically saturated with carbon dioxide. The proof of this explanation lay in the following simple test: A gas tube was filled with distilled water and 33% of carbon dioxide passed into the closed arm. This tube was placed in the incubator over night. In twelve hours all but 3% of the gas had been absorbed. Even though the liquid might become saturated and an excess of gas

be produced by some organism, the slow diffusion and escape through the open arm of the tube would result in a gradual shrinkage of gas volume. This was shown by placing 35% of carbon dioxide over water previously saturated with the gas. In thirteen days the gas volume read 15%.

The last experiment performed consisted of the innoculation of gas tubes which contained different percentages of several kinds of sugars. The sugars used were glucose, lactose, cane sugar, and maltose. The percentages used were 1%, 5%, and 10% in each case except maltose, where 1% and 5% were used. No air nor oxygen was passed into the closed arms. All the tubes were incubated at 38° C. Those containing lactose and maltose developed a very slight scum over the surface of the liquid in the open arm. The liquid in the closed arm remained perfectly clear and no gas was registered. The tubes containing 1% and 5% cane sugar developed fairly well in the open arm, but not at all in the closed arm. Where 10% cane sugar was used, ½% of gas appeared on the sixth day. The maximum percentage in this tube was 1%, acquired on the seventh day. There was an excellent development in the open arm and a fair development (cloudiness) in the closed arm.

The tubes containing glucose developed without delay. Scum formed on the surface of the liquids in a few hours. A record of the gas formation follows:

Medium Used	42 hrs.	45 hrs.	50 hrs.	53 hrs.	56 hrs.	66 hrs.	70 hrs.	74 hrs.	117 hrs.	130 hrs.
1% Glucose	3%	5%	14%	30%	48%	77%	88%	90%	30%	20%
5% Glucose	.....	2%	4%	20%	42%	100%	100%	100%	63%	63%
10% Glucose	.....	.....	.....	.....	9%	100%	100%	100%	100%	100%

At 100% the gas overflowed and bubbled out through the open arm. In the tube containing 5% glucose, a shrinkage from 100% (full) to 63% occurred, at which time evaporation of the liquid allowed air to bubble in and stop the shrinkage. The tube containing 10% glucose remained full of gas as long as observations were taken.

Food became nearly exhausted first in the tube containing 1% glucose. Diffusion and consequent loss of carbon dioxide caused shrinkage to begin first in this tube. The food in the 5% glucose tube became scarce a few hours later and shrinkage began at once. Food in the 10% glucose tube evidently was still available at the time of the last observation.

When these results are compared with those obtained from the plate cultures, we find admirable agreement. The glucose (as would probably levulose) gave very favorable conditions for quick growth and rapid gas formation. Of the disaccharides, cane sugar was the most suitable, but did not compare favorably with glucose.

Conclusions

The determination of the exact or total amounts of carbon dioxide produced by yeast is beyond the scope of this article, as are also such problems as those concerning the ease of hydrolysis of the various disaccharides by the yeast enzymes. However, from the simple experiments cited above, several concluding statements might be made:

1. That glucose and levulose cause yeast to grow much more rapidly than any of the other common sugars.

2. That glucose, (and probably levulose) causes the most rapid production of carbon dioxide.

3. That yeast grows better under aerobic conditions, but will develop in the proper medium under at least limited anaerobic conditions.

4. That in order to register gas in a gas tube, the gas must be produced in sufficient amount to more than saturate the liquid, and at a sufficient rate to overcome loss by diffusion through the open arm.

5. That a solid medium may materially alter the morphological characters of the individual yeast cells by a tendency to localize the food supply.

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EXPLANATION OF PLATES

Plate XXIII. Plate cultures on sugar agar. (1) Glucose; (2) Levulose; (3) Cane sugar; (4) Maltose; (5) Lactose; (6) Agar without sugar.

Plate XXIV. Fig. 1, Margin of surface colony on sugar agar plate. Fig. 2, Edge of deep agar colony showing filaments. Figs. 3, 4, Elongated yeast cells, showing origin, also method of branching. Fig. 5, Elongated cells, showing large vacuoles especially in older cells.